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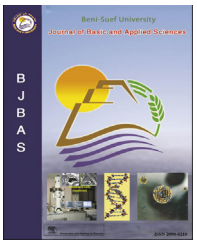

Full Length Article

Antioxidant and antibacterial activities of various extracts of *Inula cuspidata* C.B. Clarke stem

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ABSTRACT

The objective of the present study includes estimation of total phenolic and total flavonoid contents and evaluation of antioxidant and antibacterial activities of various extracts of *Inula cuspidata* stem. *I. cuspidata* belongs to family Compositae; it is an erect shrub, distributed in western Himalaya, usually found growing on steep rocky or precipitous ground.

Total phenolic and flavonoid contents were estimated by Folin–Ciocalteu and aluminum chloride method. Antioxidant activity was performed by four methods: DPPH (1,1-diphenyl-2-picryl hydrazyl radical), ferrous chelating activity, reducing power and nitric oxide scavenging activity. These extracts were screened for antibacterial studies using macro-dilution method.

Total phenolic and flavonoid contents were found to be highest in methanol extract (69.44 ± 1.12 mg GAE/g, 12.45 ± 0.67 mg QE/g) followed by chloroform (33.53 ± 0.88 mg GAE/g, 1.27 ± 0.51 mg QE/g) and *n*-hexane (12.25 ± 1.03 mg GAE/g, 0.08 ± 0.43 mg QE/g) extracts. Methanol extract of *I. cuspidata* exhibited potent antioxidant activity in all the antioxidant assays followed by chloroform and *n*-hexane extracts. IC_{50} values of methanol, chloroform and *n*-hexane extract were found to be 43.35 ± 0.58 μg/mL, 298.08 ± 0.62 μg/mL, 1989.24 ± 0.71 μg/mL for DPPH, 396.63 ± 0.73 μg/mL, 915.29 ± 0.81 μg/mL, 1180.56 ± 0.88 μg/mL for ferrous chelating and 594.68 ± 0.99 μg/mL, 930.55 ± 1.03 μg/mL, 1959.26 ± 1.25 μg/mL for nitric oxide scavenging assays. A strong correlation was found between total phenolic, flavonoid and $1/IC_{50}$ values obtained by different antioxidant assays. The correlation coefficient (R) value obtained was more than 0.9 which exhibits a strong correlation.

All the extracts showed significant antibacterial activities against Gram positive bacterial strains with minimum inhibitory concentration (MIC) values ranging from 187.5 to 750 μg/mL and moderate to weak inhibition against Gram negative bacteria with MIC values ranging from 750 to 3000 μg/mL. The present study proves the *in vitro* anti-oxidant and antibacterial activities of different extracts of *I. cuspidata* stem.

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1. Introduction

Free radicals and oxidants are of paramount importance in disease progression. They are produced either from normal cell metabolisms in situ or from external sources like pollution, cigarette smoke, radiation, and medication. Accumulation of free radicals in the body generates a phenomenon called oxidative stress, which plays a major role in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases, although the human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, but if the oxidative stress is high, the internal mechanism has to be augmented with the administration of antioxidants (PhamHuy et al., 2008).

Nowadays, natural antioxidants are gaining importance in the market as they are found to be safe, non-toxic and environmental friendly in comparison to synthetic antioxidants, which have been restricted due to their various deleterious effects (Kumaran and Karunakaran, 2007). Flavonoids, phenolic acids and tannins are considered as crucial phytoconstituents for exhibiting antioxidant activities. Redox property of hydroxyl groups present in plant phenolic is responsible for their antioxidant properties that allow them to act as hydrogen donors, reducing agents, metal chelators and free radical quenchers (Shukla et al., 2009).

In addition to free radicals developing resistance in the bacterial species to many antibiotics is another major issue in antimicrobial therapy that continuously encouraging researchers to develop novel antibiotics. Recently, most of the approved novel antimicrobials are derived from natural products or from their derivatives. According to Oshea's and Moser's analysis, out of 148 compounds 66% fall into natural product category (Brown et al., 2014). Thus, natural products are of paramount importance as a source of antibacterial agents. The antibacterial activity is mainly associated with the presence of secondary metabolites like phenolic, terpenes and alkaloids present in the plant extracts.

The genus *Inula*, a variable perennial herb distributed in Asian, African and European continents, comprises more than hundred species of the Compositae (Asteraceae) family belonging to the tribe Inuleae (Seca et al., 2014; Zhao et al., 2006). *Inula* species possess medicinal properties and are used in folk medicines as tonic, stomachic, anti-inflammatory, bactericidal, diuretic, diaphoretic, hepatoprotective, antitumor and carminative (Mathela et al., 2008). Leave extract of *Inula cuspidata* shows anti-inflammatory, antifungal and antibacterial activities (Chauhan and Saxena, 1985; Sati et al., 2011; Thapliyal et al., 2011) and stem, flower and whole plant extracts were reported to have profound anti-inflammatory and hepatoprotective activities (Kaur et al., 2014a, 2014b). Previous chemical investigations done on the *I. cuspidata* shows the presence of monoterpenoids, sesquiterpenoids, flavonoids and glycosides (Bohlmann et al., 1982; Sahai et al., 1981; Verma et al., 2014). Until today, this plant has not been explored for its quantitative property (total phenol, total flavonoid content) and antioxidant activities; thus, the aim of the study was to evaluate the phyto-quantitative property and antioxidant and antibacterial activities of different stem extracts of *Inula cuspidata*. Results from this work will enlighten the medicinal aspects of this herb.

2. Materials and methods

2.1. Collection and authentication of plant material

Stems of *I. cuspidata* were procured from Nainital, Uttarakhand, India, in the months of August to September 2013 and were authenticated (Voucher number-114758) at Botanical Survey of India, Dehradun. The stems were shade dried, coarsely powdered and stored in an air tight container for further use.

2.2. Chemicals, reagents and instrument

L-Ascorbic acid, quercetin, gallic acid, trichloroacetic acid, DPPH, ferrozine and nutrient broth were purchased from Himedia, Mumbai, India. Sulfanilamide, EDTA and N-1-napthyl ethylene diamine dihydrochloride were purchased from Sigma Aldrich, Mumbai, India. Folin–Ciocalteu reagent, potassium ferricyanide, ferrous chloride, and ferric chloride were procured from Merck, Mumbai, India. Aluminum chloride and dimethyl sulfoxide was procured from Molychem, Mumbai, India. All the chemicals used in the study were of analytical grade. The rotary evaporator was procured from Heidolph, Schwabach, Germany. UV–visible spectrophotometer (Shimadzu, UV-1800) was purchased from Shimadzu, Japan.

2.3. Physico-chemical evaluation

The stem powder was evaluated for physico-chemical parameters such as total ash value, acid insoluble ash value, water soluble ash value and alcohol soluble extractive value as per WHO guidelines (WHO, 1998).

2.4. Extraction of plant material

2.4.1. Maceration method

The coarsely powdered plant material (20 g) was taken in a glass-stoppered conical flask and extracted thrice with organic solvent (3 × 100 mL) in a sequential manner first with *n*-hexane followed by chloroform and methanol. The extraction was carried out in a mechanical shaker at room temperature for 6 h and then allowed to stand for 18 h. The extraction time for each solvent was about 3 × 24 h respectively. The extracts obtained were filtered and concentrated under vacuum using rotary evaporator (Heidolph, Schwabach, Germany). Yields of extracts were calculated on the basis of percentage w/w (WHO, 1998).

2.5. Preliminary phytochemical screening

The preliminary phytochemical screening was performed for identifying the presence of phyto constituents in *n*-hexane, chloroform and methanol extracts of *I. cuspidata* stem (Harborne, 1998).

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2.6. Estimation of total phenolic content by colorimetric method

The total phenolic content present in the plant extracts was determined by using the method reported by Singleton et al. (1999). To the plant extract (1 mL, 1000 μg/mL), Folin–Ciocalteu reagent (1.5 mL) was added, mixed and kept aside at room temperature for 5 min. Sodium carbonate (4 mL, 7.5%) and distilled water (3.5 mL) were added to the reaction mixture and allowed to stand at room temperature for 30 min. The absorbance of the reaction mixture was measured at 765 nm using a UV–visible spectrophotometer. Gallic acid (20–100 μg/mL) was used as standard. Total phenolic content was calculated from the standard plot of gallic acid and expressed as mg gallic acid equivalents per gram of dry weight of extract (mg GAE/g).

2.7. Estimation of total flavonoid content by colorimetric method

The total flavonoid content present in the plant extracts was determined by using the method reported by Chang et al. (2002). To the plant extract (1 mL, 1000 μg/mL), methanol (3 mL), aluminum chloride (0.2 mL, 10%), potassium acetate (0.2 mL, 1 M) and distilled water (5.6 mL) were added. The reaction mixture was incubated at room temperature for 30 min and the absorbance was measured at 415 nm using a UV–visible spectrophotometer. Quercetin (10–50 μg/mL) was used as standard. Total flavonoid content was calculated from the standard plot of quercetin and expressed as quercetin equivalents per gram of dry weight of extract (mg QE/g).

2.8. In vitro antioxidant studies

2.8.1. DPPH assay

The free radical scavenging activity of plant extracts was determined by DPPH (1,1-diphenyl-2-picryl hydrazyl) assay using the method reported by Gursoy et al. (2009) with slight modifications. Two milliliters of DPPH radical solution (50 μM in methanol) was added to 2 mL each of various concentrations of plant extracts ranging from 20 to 2000 μg/mL and standard ascorbic acid (2–10 μg/mL). The reaction mixtures were shaken thoroughly and incubated in the dark for 30 min. The absorbance of all the resulting solutions was measured at 517 nm using a UV–visible spectrophotometer. Percentage inhibition was calculated by the following equation:

% Inhibition = $(A_0 - A_1)/A_0 \times 100$, where A_0 is the absorbance of control solution (contains all the test reagents except the test compound) and A_1 is the absorbance of plant extracts or standard.

2.8.2. Reducing power assay

The reducing power of the plant extracts was determined by using the method reported by Hseu et al. (2008). 1 mL of each extract (200–4000 μg /mL) and standard ascorbic acid (10–50 μg/mL) were mixed with phosphate buffer solution (2.5 mL, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The solutions were mixed and incubated at 50 °C for 20 min. After incubation, trichloroacetic acid (2.5 mL, 10%) was added to each reaction mixture and then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and allowed to stand for 10 min. The absorbance was measured at 700 nm.

2.8.3. Ferrous chelating activity

The metal chelating activity of plant extracts was studied by the method described by Dinis et al. (1994). To 1 mL of each extract (200–2000 μg/mL) and standard EDTA (5–25 μg/mL), ferrous chloride (0.2 mL, 2 mM) and ferrozine (0.4 mL, 5 mM) were added. The total volume was adjusted to 4 mL with methanol, then the reaction mixtures were shaken vigorously and allowed to stand at room temperature for 10 min. The absorbance of the reaction mixtures was measured at 562 nm. The ferrous chelating activity was calculated by using the equation: Chelating activity (%) = $(A_0 - A_1)/A_0 \times 100$, where A_0 is the absorbance of control solution (contains all the test reagents except the test compound) and A_1 is the absorbance of plant extracts or standard.

2.8.4. Nitric oxide scavenging activity

The nitric oxide scavenging activity of plant extracts was studied by using the method reported by Chanda and Dave (2009). To 2 mL aliquot of different dilutions of extracts (200–2000 μg/mL) and standard ascorbic acid (100–500 μg/mL), sodium nitroprusside (3.0 mL) prepared in phosphate buffered saline (10 mM) was added and incubated at 25 °C for 2 h. After incubation 2.0 mL of reaction mixture was withdrawn and mixed with 2 mL of Griess reagent (1% sulfanilamide, 2% orthophosphoric acid and 0.1% N-1-napthyl ethylene diamine dihydrochloride). The absorbance of pink color chromophore was recorded at 546 nm. The nitric oxide scavenging activity was calculated by using the equation: Nitric oxide scavenging activity (%) = $(A_0 - A_1)/A_0 \times 100$, where A_0 is the absorbance of control solution (contains all the test reagents except the test compound) and A_1 is the absorbance of plant extracts or standard.

2.9. Anti-bacterial activity

Minimum inhibitory concentrations (MIC) against four pathogenic bacterial strains were determined using the macro dilution method. The organisms tested were two Gram positive bacteria – *Staphylococcus aureus* and *Bacillus subtilis* – and two Gram negative bacteria – *Escherichia coli* and *Pseudomonas aeruginosa*. The serial dilutions of plant extracts (from 3000 to 93.75 μg/mL) and standard ampicillin (from 100 to 3.125 μg/mL) for each bacteria were prepared in nutrient broth in sterilized test tubes, then bacterial inoculum (10^6 CFU/mL) was added to each test tube. The control tube containing bacterial inoculum and nutrient broth was prepared for each bacteria. The tubes were incubated at 37 °C for 24 h. After incubation, all the test tubes containing plant extracts and standard were compared with the control for the determination of bacterial growth inhibition. Further, MIC value for each plant extract/standard was determined (Mazurova et al., 2015). All the tests were performed in triplicate and MIC values were found to be constant.

2.10. Statistical analysis

The experimental results were expressed as mean ± SD of three parallel measurements. Correlation coefficient (R) between $1/IC_{50}$

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values and total phenolic and flavonoids was calculated by using GraphPad Prism version 5.03 software.

3. Results and discussion

The present study manifests physicochemical parameters, quantification of total phenolic and flavonoids, and investigation of antioxidant and antibacterial activities of various extracts of *I. cuspidata* stem.

3.1. *Physico-chemical evaluation, extraction method and preliminary phytochemical screening*

Ash values represent the presence of inorganic content in the drug and also determine the purity and quality of drug (WHO, 1998; Urmila, 2012). The outcome of physico-chemical studies of the drug like ash value and extractive values is given in Table 1. Extractive value determines the amount of active constituents extracted by the particular solvent from a particular amount of the drug (WHO, 1998). Extractive value helps to indicate the nature of chemical constituents present in the drug and also useful in estimation of specific constituents soluble in a particular solvent. Extractive values are primarily useful for the determination of exhausted or adulterated drugs (Kumar et al., 2011). The alcohol soluble extractive value was found to be 7.25 ± 0.28% which revealed the presence of alcohol soluble phyto-constituents such as alkaloids, flavonoid, phenolic, terpenoids and steroids in the stem part of *I. cuspidata*. The maceration method was adopted for extraction of *I. cuspidata* stem and sequential extraction was performed by using the solvents of different polarity. The percentage yield of extract obtained depends mainly on the solubility of the phyto-constituents present in the plant material into a suitable solvent (Patil et al., 2012). The non-polar constituents are soluble in *n*-hexane or petroleum ether, while medial polar constituents are dissolved in chloroform and highly polar constituents are soluble in methanol. The yield of the different extracts obtained by maceration is represented in Table 2. The percentage yield of all the extracts was found to be in the order of methanol (4.29 ± 0.42% w/w) > *n*-hexane (1.38 ± 0.35% w/w) > chloroform (0.92 ± 0.21% w/w). The results obtained in the study revealed that the stem part of *I. cuspidata* is enriched in polar constituents and also it possesses a good amount of non-polar constituents as well. Preliminary phytochemical screening results revealed the presence of alkaloids, steroids, triterpenoids, phenolic, flavonoids, proteins, and carbohydrates in different extracts of *I. cuspidata* stem (Table 3). The presence of secondary metabolites is prerequisite for various biological and pharmacological activities.

Table 1 – Physico-chemical evaluation of stem of *Inula cuspidata*.

Physico-chemical parameter	Observed values (% w/w)
Total ash	4.48 ± 0.28
Acid insoluble ash	0.5 ± 0.46
Water soluble ash	1.51 ± 0.58
Alcohol soluble extractive value	7.25 ± 0.28

Values are expressed as mean ± SD, n = 3.

Table 2 – Percentage yield of different extracts of stem of *Inula cuspidata*.

Type of extract	Extraction method	Color	% Yield
n-Hexane extract	Maceration	Light yellowish brown	1.38 ± 0.35
Chloroform extract	Maceration	Brown	0.92 ± 0.21
Methanol extract	Maceration	Dark brown	4.29 ± 0.42

Values are expressed as mean ± SD, n = 3.

3.2. *Total phenolic and flavonoid content analysis*

Phenolic compounds or polyphenols are ubiquitously distributed class of plant secondary metabolites, with more than 8000 phenolic structures have been reported yet. The most described property of phenolic is their antioxidant and free radical scavenging activity (Fu et al., 2012; Leopoldini et al., 2011). In this study, the total phenolic of all the extracts was estimated by Folin–Ciocalteu reagent and the results are shown in Table 4. The total phenol content was expressed in gallic acid equivalents (GAE) and calculated from linear regression equation of standard curve ($y = 0.0093x - 0.0268$, $R^2 = 0.9993$). The highest amount of total phenolic (69.44 ± 1.12 mg GAE/g) was obtained in methanol extract, followed by chloroform (33.53 ± 0.88 mg GAE/g) and *n*-hexane extract (12.25 ± 1.03 mg GAE/g). The results revealed that the methanol extract contains the highest total phenolic content, and thus it possesses better antioxidant potential than the other extracts. Phenolic constituents precipitate antioxidant activities by undergoing

Table 3 – Preliminary phytochemical screening of different extracts of stem of *Inula cuspidata*.

Phytochemical test	*n*-Hexane extract	Chloroform extract	Methanol extract
Alkaloids	–	–	+
Carbohydrates	–	–	+
Flavonoids	–	–	+
Triterpenoids	+	+	–
Steroids	+	+	+
Phenolic	+	+	++
Proteins and amino acids	–	–	+
Saponins	–	–	–

+ = present; – = absent.

Table 4 – Total phenolic and flavonoid contents of different extracts of stem of *Inula cuspidata*.

Plant extract	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)
n-Hexane	12.25 ± 1.03	0.08 ± 0.43
Chloroform	33.53 ± 0.88	1.27 ± 0.51
Methanol	69.44 ± 1.12	12.45 ± 0.67

Values are expressed as mean ± SD, n = 3.

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Table 5 – DPPH radical scavenging activity of different extracts of stem of *Inula cuspidata*.

Ascorbic acid		*n*-Hexane extract		Chloroform extract		Methanol extract	
Conc. (µg/mL)	Percent inhibition	Conc. (µg/mL)	Percent inhibition	Conc. (µg/mL)	Percent inhibition	Conc. (µg/mL)	Percent inhibition
2	8.45 ± 0.38	500	14.7 ± 0.62	100	22.62 ± 0.52	20	32.51 ± 0.42
4	23.25 ± 0.42	1000	22.41 ± 0.72	200	35.87 ± 0.66	40	48.39 ± 0.59
6	38.86 ± 0.82	1500	36.83 ± 1.05	300	51.29 ± 0.98	60	62.19 ± 0.73
8	55.93 ± 0.63	2000	51.67 ± 0.96	400	65.99 ± 1.14	80	76.18 ± 0.61
10	68.78 ± 0.72			500	75.5 ± 0.73	100	85.06 ± 0.82

Values are expressed as mean ± SD, n = 3.

a redox reaction with the phosphotungstate and phosphomolybdate present in the Folin–Ciocalteu reagent. However, this assay is not a specific method for the estimation of phenolic (Wong et al., 2006). Many researchers have reported that this method also gives results for those substances which could be oxidized by Folin reagent. Although, this method could give an idea about the crude estimate of phenolic compounds present in different extracts of stem of *I. cuspidata*.

Flavonoids exhibit antioxidant activity by different mechanisms which include direct scavenging of reactive oxygen species, metal chelation, inhibition of oxidases and activation of antioxidant enzymes (Prochazkova et al., 2011). The total flavonoid content was determined by aluminum chloride colorimetric method and results are summarized in Table 4. The total flavonoid content was expressed in quercetin equivalents (QE) and calculated from linear regression equation of standard curve ($y = 0.0105x + 0.0253$, $R^2 = 0.9993$). The total flavonoid content in different extracts decreased in the following order: methanol extract (12.45 ± 0.67 mg QE/g) > chloroform extract (1.27 ± 0.51 mg QE/g) > *n*-hexane (0.08 ± 0.43 mg QE/g).

3.3. *Antioxidant activity*

3.3.1. *DPPH assay*

DPPH free radical scavenging assay is one of the most widely used methods in the determination of antioxidant activity of crude extracts and pure compounds. DPPH is a stable free radical, produces violet color in methanol and on reaction with a hydrogen donating antioxidant, DPPH free radical is reduced to DPPH-H; this leads to the discoloration of DPPH solution from violet to yellow (Tuba and Gulcin, 2008). The DPPH free radical scavenging activities of various extracts are shown in Table 5. The IC_{50} value of all the extracts was determined, which depicted the concentration of the extracts required to scavenge 50% of DPPH free radical. Low IC_{50} value represents high antioxidant potential. As shown in Fig. 1, IC_{50} value of methanol extract was found to be less than 43.35 ± 0.58 µg/mL, as compared to IC_{50} values of chloroform and *n*-hexane extracts which were found to be 298.08 ± 0.62 µg/mL and 1989.24 ± 0.71 µg/mL, while IC_{50} value of standard ascorbic acid was found to be 7.43 ± 0.52 µg/mL. This proves that methanol extract possesses high free radical scavenging activity as compared to chloroform and *n*-hexane extracts. The study established a positive and strong correlation between antioxidant activity and total phenolic as well as total flavonoid content. The methanol extract revealed high phenolic and flavonoid content results in good antioxidant potential.

3.3.2. *Reducing power assay*

The reducing power assay is based on the measurement of reducing potential of crude extract or compound. The reducing power ability (electron donating capacity) is based on the transformation of ferric to ferrous ion in the presence of crude extract

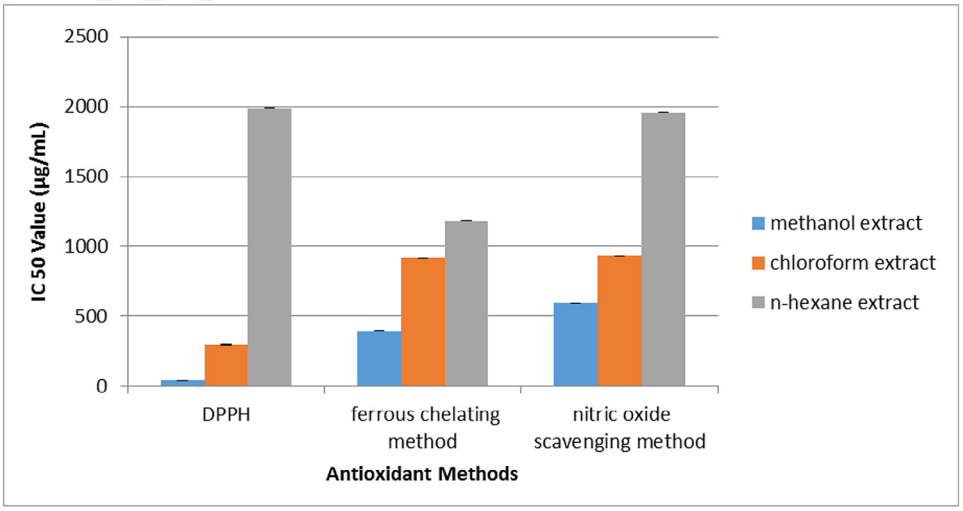

Fig. 1 – IC_{50} values of different extracts of stem of *Inula cuspidata*.

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Table 6 – Reducing power of different extracts of stem of *Inula cuspidata*.

Ascorbic acid		*n*-Hexane extract		Chloroform extract		Methanol extract	
Conc. (μg/mL)	Percent reducing power	Conc. (μg/mL)	Percent reducing power	Conc. (μg/mL)	Percent reducing power	Conc. (μg/mL)	Percent reducing power
10	26.6 ± 0.61	1000	16.8 ± 0.81	500	26.6 ± 0.74	200	25.4 ± 0.64
20	35.7 ± 0.69	2000	25.4 ± 0.74	750	38.0 ± 0.99	400	49.0 ± 0.52
30	47.5 ± 0.57	3000	38.3 ± 1.25	1000	55.2 ± 1.06	600	57.8 ± 0.84
40	56.9 ± 0.68	4000	44.0 ± 1.12	1250	63.9 ± 1.14	800	71.9 ± 0.99
50	67.1 ± 0.88					1000	92.2 ± 0.98

Values are expressed as mean ± SD, n = 3.

or compound (Oliveira et al., 2008). It was suggested that the electron donating capacity is associated with antioxidant activity (Tuba and Gulcin, 2008). The reducing power ability of various extracts and ascorbic acid is summarized in Table 6. Reducing power of standard ascorbic acid and different extracts exhibited the following order: ascorbic acid > methanol extract > chloroform extract > *n*-hexane extract. The results depicted that maximum reducing power (92.2 ± 0.98%) was exhibited by the methanol extract at the concentration of 1000 μg/mL, whereas at the same concentration reducing power exhibited by chloroform and *n*-hexane extracts were found to be 55.2 ± 1.06% and 16.8 ± 0.81%. The maximum reducing power showed by ascorbic acid was 67.1 ± 0.88% at the concentration of 50 μg/mL.

3.3.3. *Ferrous chelating assay*

The antioxidant potency of an extract is determined by its metal chelating power. Metal ions such as ferrous ion can facilitate the production of reactive oxygen species. Ferrous ion mainly involves in Fenton reaction where it reacts with hydrogen peroxide to generate the most reactive and destructive reactive oxygen species hydroxyl radical (Aslani and Ghobadi, 2016). Thus the metal chelating power is also associated with antioxidant activity. The metal chelating power of various extracts and EDTA is summarized in Table 7. The results showed that metal chelating power of all the extracts and standard was concentration dependent. The metal chelating power decreased in the order: EDTA > methanol extract > chloroform extract > *n*-hexane extract. The IC_{50} value (Fig. 1) of methanol extract was found to be 396.63 ± 0.73 μg/mL, whereas for chloroform and *n*-hexane extracts IC_{50} values were found to be 915.29 ± 0.81 μg/mL and 1180.56 ± 0.88 μg/mL. The standard EDTA exhibited maximum metal chelating power with IC_{50} value of 16.64 ± 0.48 μg/mL. Flavonoids contain phenolic groups that have the ability to chelate metal ions in order to prevent their participation in free radical generation and proliferation. The present study showed a strong correlation between metal chelating power and total phenolic as well as flavonoid content. The methanol extract which contained highest phenolic and flavonoid content exhibited maximum metal chelating power than other extracts.

3.3.4. *Nitric oxide scavenging assay*

Nitric oxide (NO) plays a key role in the pathogenesis of inflammation (Kalidindi et al., 2015; Omer et al., 2012; Sharma et al., 2007; Umamaheswari and Chatterjee, 2008). Inflammatory disease is associated with increased production of nitric oxide (NO) and activation of the inducible nitric oxide synthase (iNOS) pathway (Omer et al., 2012). The nitric oxide scavenging ability of all the extracts is shown in Table 8. The findings suggested that nitric oxide scavenging power of all extracts was concentration dependent. Among all the extracts methanol was found to possess maximum nitric oxide radical scavenging activity followed by chloroform and *n*-hexane extracts. The methanol extract exhibited maximum nitric oxide inhibition (75.52 ± 1.69%) at the concentration of 1000 μg/mL, whereas the nitric oxide inhibition by chloroform and *n*-hexane extracts at the same concentration were found to be 53.88 ± 1.45% and 23.27 ± 1.31%. The IC_{50} value obtained for methanol extract was 594.68 ± 0.99 μg/mL, chloroform extract was 930.55 ± 1.03 μg/mL, *n*-hexane extract was 1959.26 ± 1.25 μg/mL and ascorbic acid was 284.9 ± 0.88 μg/mL. The linear correlation was obtained between nitric oxide scavenging activity and total phenolic as well as flavonoid content.

To the best of our knowledge this is the first report on estimation of total phenolic and total flavonoid content, as well as on evaluation of antioxidant activity of *I. cuspidata*. The find-

Table 7 – Ferrous chelating activity of different extracts of stem of *Inula cuspidata*.

EDTA		*n*-Hexane extract		Chloroform extract		Methanol extract	
Conc. (μg/mL)	Percent inhibition	Conc. (μg/mL)	Percent inhibition	Conc. (μg/mL)	Percent inhibition	Conc. (μg/mL)	Percent inhibition
5	11.6 ± 0.48	500	22.5 ± 0.74	500	20.52 ± 0.66	200	35.6 ± 0.55
10	28.65 ± 0.62	1000	41.78 ± 1.03	750	41.36 ± 0.78	400	50.53 ± 1.04
15	41.99 ± 0.37	1500	67.32 ± 1.14	1000	54.65 ± 0.99	600	66.26 ± 0.84
20	52.43 ± 0.56	2000	79.1 ± 0.99	1250	75.81 ± 1.25	800	75.6 ± 0.73
25	87.35 ± 0.52			1500	85.55 ± 0.82	1000	81.6 ± 1.03

Values are expressed as mean ± SD, n = 3.

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Table 8 – Nitric oxide scavenging activity of different extracts of stem of *Inula cuspidata*.

Ascorbic acid		*n*-Hexane extract		Chloroform extract		Methanol extract
Conc. (μg/mL)	Percent inhibition	Conc. (μg/mL)	Percent inhibition	Conc. (μg/mL)	Percent inhibition	Percent inhibition
100	25.0 ± 0.72	500	11.74 ± 1.12	200	10.71 ± 0.81	25.91 ± 1.11
200	39.92 ± 0.84	1000	23.27 ± 1.31	400	22.45 ± 1.11	36.26 ± 0.88
300	55.75 ± 1.12	1500	36.22 ± 2.11	600	29.79 ± 1.61	51.7 ± 1.25
400	60.97 ± 0.93	2000	52.55 ± 1.84	800	44.49 ± 1.38	62.17 ± 1.31
500	69.78 ± 1.03			1000	53.88 ± 1.45	75.52 ± 1.69

Values are expressed as mean ± SD, n = 3.

ings of antioxidant study were found consistent with the previously published reports on the other species of the genus *Inula* (AL-Fartosy, 2011; Chahmi et al., 2015; Jallali et al., 2014; Mahmoudi et al., 2016).

3.4. *Correlation of 1/IC_{50} values of different antioxidant methods with total phenolic and flavonoid contents*

The correlation coefficients (R) between total phenolic and 1/IC_{50} values for DPPH, ferric chelating and nitric oxide scavenging were found to be 0.966193, 0.97046 and 0.992571 (Table 9). As R value found in this study was high, it indicates that there is a strong correlation between total phenolic content and antioxidant activity. The outcomes of the study are in accordance with the previously published findings of scientist/authors, who mentioned a linear correlation between the total phenolic content and antioxidant activity in their studies (Li et al., 2009). Similarly, a strong correlation was also drawn between total flavonoid and 1/IC_{50} values for DPPH, ferrous chelating and nitric oxide scavenging assays and is presented in Table 9 with R values of 0.999582, 0.998942 and 0.916469 respectively. The outcomes of the study are in good agreement with the previous findings, where the authors represented a significant and linear correlation between total flavonoid content and antioxidant activity (Wen et al., 2012).

Table 9 – Correlation between 1/IC_{50} values of antioxidant assays and total phenolic and flavonoid contents.

Correlation coefficient (R)	Total phenolic	Total flavonoid
DPPH assay	0.966193	0.999582
Ferrous chelating assay	0.97046	0.998942
Nitric oxide scavenging assay	0.992571	0.916469

3.5. *Antibacterial activity*

Different extracts of *I. cuspidata* stem were evaluated for their antibacterial activities using the macro dilution method. The results of this study are summarized in Table 10. All the extracts exhibited significant antibacterial activity against Gram positive bacterial strains. The *n*-hexane extract showed effective antibacterial activity against Gram positive bacteria. The MIC value (187.5 μg/mL) of *n*-hexane extract obtained for *B. subtilis* was found to be significant as compared to other extracts whereas MIC value for *S. aureus* (375 μg/mL) was found to be good and was similar to that of methanol extract. The *n*-hexane extract showed moderate antibacterial activity against Gram negative bacteria *E. coli* with MIC value of 750 μg/mL. However, *n*-hexane extract exhibited weak antibacterial activity against *P. aeruginosa* with MIC value of 1500 μg/mL. Methanol extract showed good antibacterial activity against Gram positive bacteria and moderate antibacterial activity against Gram negative bacterial strains. Methanol extract exhibited same MIC value (375 μg/mL) for *B. subtilis* and *S. aureus* bacterial strains. However, the methanol extract showed MIC value of 750 μg/mL for both *E. coli* and *P. aeruginosa*. The chloroform extract showed good to moderate antibacterial activity against Gram positive bacteria and weak antibacterial activity against Gram negative bacterial strains. MIC values of chloroform extract were found to be 375 μg/mL for *B. subtilis* and 750 μg/mL for *S. aureus* whereas the MIC values of chloroform extract for *E. coli* and *P. aeruginosa* were found to be 1500 μg/mL and 3000 μg/mL.

It is noteworthy that MIC values obtained for the plant extracts were found to be more than that of standard drug ampicillin against both Gram positive and negative bacteria. The results showed that Gram positive bacteria were more susceptible to the extracts than Gram negative bacteria. These results are in agreement with the earlier studies done by our team on *I. cuspidata* leaves (Sati et al., 2011) which indicates that the stem part is also susceptible toward Gram positive

Table 10 – MIC of different extracts of stem of *Inula cuspidata*.

Plant extracts/Control	MIC (μg/mL)			
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
n-Hexane	187.5	375	750	1500
Chloroform	375	750	1500	3000
Methanol	375	375	750	750
Ampicillin	6.25	12.25	25	100

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bacteria. In addition, the results are also in accordance with that of previous studies done on other species of *Inula* (Gokbulut et al., 2013). The *n*-hexane and chloroform extracts showed lower sensitivity toward Gram negative bacteria and this could be due to the presence of lipopolysaccharides in the outer membrane of Gram negative bacteria, which hinders the diffusion of hydrophobic compounds and thus protects the bacterial cell membrane from damage.

The antibacterial activity of plant extracts depends on the phyto-constituent composition, type of solvent chosen for the extraction, extent of miscibility of antibacterial compounds in the particular solvent, solubility of antibacterial components in test medium and the method chosen for the evaluation (Ahmed et al., 2014).

The study revealed that antibacterial components are effectively concentrated in the non-polar fraction against the Gram positive bacteria whereas the polar fraction was enriched in such phyto-constituents which showed antibacterial activity against Gram negative bacteria. The antibacterial activity exhibited by the extracts could be due to the presence of secondary metabolites, which may act alone or synergistically for the inhibition of bacterial growth (Naidu et al., 2013).

4. Conclusion

The different stem extracts of *I. cuspidata* were studied for their total phenolic and total flavonoid contents in addition to antioxidant and antibacterial potential. All the extracts showed significant antioxidant activities in different concentrations. Methanol extract exhibited highest antioxidant activity as well as more phenolic and flavonoid contents in comparison with other extracts. A positive correlation was found between antioxidant activity and total phenolic content as well as total flavonoid content. All the extracts exhibited effective antibacterial activity against Gram positive bacteria and moderate to weak inhibition against Gram negative bacteria. To the best of our knowledge antioxidant activity and total phenolic and flavonoid contents have not been studied before for *I. cuspidata*. Further research work will focus on the isolation and characterization of secondary metabolites responsible for antioxidant and antibacterial activities.

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